MICROFLUIDIZED LEISHMANIA LYSATE AND METHODS OF MAKING AND USING THEREOF

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

[01] This invention was made by employees of the United States Army. The government has rights in the invention.

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION.

[02] This invention relates generally to microfluidized *Leishmania* lysate preparations. In particular, the present invention relates to microfluidized *Leishmania* lysate preparations for assays and immunogenic compositions.

2. DESCRIPTION OF THE RELATED ART.

[03] Leishmaniasis is a serious and sometimes fatal disease. The World Health Organization (WHO) reports that about 40 million individuals are infected with *Leishmania* in 88 countries. Transmission of leishmaniasis is especially problematic in Mediterranean Africa, Asia, and Latin America. Leishmaniasis is a threat individuals who travel to or live in endemic areas. There are about 10 to about 25 new cases of cutaneous leishmaniasis in U.S. military personnel each year. There have been some notable outbreaks of leishmaniasis which yielded about 50 cases per year and attach rates as high as 50% in military personnel. There is one instance when Canadian Paratroopers suffered an attack rate of over 90% in as few as 6 hours of being in Leishmania "hot spots" in French Guyana. Additionally, in 1991, the Gulf War presented a new clinical syndrome of visceral leishmaniasis caused by *L. tropica*. *See* Magill, A.J. *et al.* (1993) N. Engl. J. Med. 328(19):1383-1387; and Magill, A.J. *et al.* (1994) Clin. Infect. Dis. 19(4):805-806.

[04] Unfortunately, current acceptable diagnostic practices lack the means for efficiently and accurately identifying those infected or exposed to the disease-causing parasite as the majority of Leishmania infections do not result in overt clinical manifestations. *See* Pampiglione, S. *et al.* (1974) Trans. Roy. Soc. Trop. Med. Hyg. 68(6):447-453; Ho, M. *et al.* (1982) Trans. Roy. Soc. Trop. Med. Hyg. 76(6):741-746; and Evans, T. *et al.* (1992) J. Infect. Dis. 166:1124-1132. There are many assays

designed to detect a cell-mediated immune response in exposed individuals. However, these test are costly, time consuming and hard to perform. In addition, prior art methods for detecting cell-mediated immunity against leishmanial antigens, such as T-cell proliferation and cytokine production, are not only difficult to standardize, but they require the collection and cyropreservation of peripheral blood mononuclear cells. For these reasons present assays design to detect cell-mediated responses are not technically or logistically practical for screening large numbers of individuals.

[05] Serology, primary ELISA, IFA and agglutination assays are somewhat useful in the diagnosis of visceral leishmaniasis. Nevertheless, these assays are of little use for diagnosing cutaneous and mucocutaneous leishmaniasis where the antibody titers are low. As a result, the prevention of leishmanial epidemics is greatly hindered and patient management is difficult.

[06] Although there are many prior art methods for detecting cell-mediated immunity against leishmanial antigens, such as T-cell proliferation and cytokine production, the prior art methods are not only difficult to standardize, but they require the collection and cyropreservation of peripheral blood mononuclear cells and are not technically or logistically practical for screening large numbers of individuals.

[07] Skin test assays are a practical way to screen large numbers of individuals as delayed hypersensitivity occurs with most individuals having cutaneous, mucocutaneous, post kala-azar dermal, and cured visceral leishmaniasis may be measured by the leishmanin test. The use of crude leishmanial antigens to elicit DTH was first reported by Montenegro in 1926. *See* Montenegro, J. (1926) Archives Dermatology and Syphilology 13187-194. Since then, many different leishmanial preparations for skin tests have been prepared and used in endemic areas. Most employ a locally acquired strain of *Leishmania* and make a crude antigenic preparation comprising either whole promastigotes or disrupted promastigotes (sonicated or freeze-thawed) or soluble antigens.

[08] These leishmanial preparations are problematic as the preparations suffer from lack of standardization, undefined sensitivity and specificity, unknown sensitizing capacity, and unknown dose or response relationships between antigen content, clinical syndrome, or infecting parasite. Further, the prior art preparations have short-term shelf life. For example, Reed *et al.* discloses a leishmanial skin test antigen that is safe and effective. *See* Reed, S. *et al.* (1986) Am. J. Trop. Med. Hyg. 35:79-85. SDS-PAGE of a

fresh preparation provided a series of distinct bands; however, SDS-PAGE of an older stored batch did not provide distinct bands thereby indicating protein degradation by proteases. Other problems of leishmanin tests include the lack of a universal and standardized Leishmania skin test antigen which may be used to set the standard of care or diagnosis in various countries. These problems of the prior art preparations and methods have prevented development and approval of a leishmanial skin test antigen for clinical use.

[09] In summary, the strengths of the leishmanin tests include delayed hypersensitivity as an important feature of all forms of leishmaniasis that may be measured and that the parasitic antigens elicit a cutaneous delayed-type hypersensitivity response in most individuals with cutaneous leishmaniasis, mucocutaneous leishmaniasis, post kala-azar dermal leishmaniasis, and cured visceral leishmaniasis. The shortcomings of the leishmanin tests include false positive reactions that can be high in areas where there is a background of leishmaniasis, as many individuals in the healthy populations of endemic leishmaniasis areas having no evidence of past infection may show high rates of leishmanin sensitivity. The shortcomings also include cross-reactions with cases of glandular tuberculosis and lepromatous leprosy, cross-reactivity between Leishmania strains as heterologous antigens often give smaller reactions, lack of a universal and standardized Leishmania skin test antigen, unknown sensitizing capacity, unknown stability, unknown dose/response relationships between antigen content and clinical syndrome or infecting parasite, and the standard of care of using Leishmania skin test antigen in many endemic countries is not approved by drug approval agencies such as the U.S. Food and Drug Administration.

[10] Thus, a need still exists for an effective, convenient, simple, and cost effective assay to detect cell-mediated immunity against leishmanial antigens that renders itself to be manufactured following cGMP so it can be approved by the FDA.

SUMMARY OF THE INVENTION

[11] The present invention generally relates to microfluidized leishmanial antigens and methods of making and using thereof.

[12] In some embodiments, the present invention relates to a method of preparing a microfluidized lysate preparation comprising microfluidizing a slurry of at least one *Leishmania* parasite through a chamber and disrupting the leishmania parasite with a